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Rats with electrodes chronically implanted in the perforant path for electrical stimulation, and dentate gyrus for recording were treated with a single oral administration of either saline, 5 mg/kg trimethyltin (TMT) or 6 mg/kg TMT. Recurrent inhibition was assessed by paired pulse activation of the perforant path input to the dentate gyrus. The measure of recurrent inhibition employed was the ratio of the population spike amplitudes of the responses to the first and second of the paired stimuli. Inhibition was assessed immediately before, and at 2, 24 and 120 hrs following TMT. The results indicated a reduction in inhibition as early as 2 hr following treatment, suggesting that TMT-induced destruction of hippocampal pyramidal cells may be secondary to their over-activation from an uninhibited mossy fiber system.

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Trimethyltin Reduces Recurrent Inhibition in Rats[1]

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Trimethyltin TMT Hippocampal evoked potentials Recurrent inhibition Paired pulse paradigm
Dentate gyrus Basket cells

ACUTE oral administration of trimethyltin (TMT) to adult rats produces widespread morphological, behavioral and physiological alterations [4]. The pattern of morphological damage is in some respects similar to the pattern of damage produced by intraventricular kainic acid [8]. Cellular necrosis is extensive in the pyramidal cells of the hippocampal formation, appearing to involve first CA3c, then CA3b and CA3a [3,5].

The mechanism by which TMT produces this damage is unknown. However a similar pattern of damage has been produced by repetitive electrical stimulation of the mossy fiber system at intensities and durations below those required to elicit afterdischarges [9]. Therefore if TMT increased activity in the mossy fiber system, damage to the hippocampal pyramidal cells could result.

Theoretically, activity in the mossy fibers could be increased by reduction of recurrent inhibition in the basket cell-granule cell system. The purpose of the present study was to test the hypothesis that recurrent inhibition in this pathway is decreased in rats treated with TMT.

METHOD

Adult (60 day old) Long-Evans hooded rats (n=36) were obtained from Charles River Breeding Co. and housed individually in plastic cages on wood chip bedding in a room maintained at 22°C and with a light:dark cycle of 12:12. The rats were implanted with a twisted bipolar 0.1 mm nichrome wire (insulated except at the cut tips) stimulating electrode in the perforant path (pp) (−6.6 mm from bregma, 3.7 mm lateral and 3.4 below the cortical surface) and a monopolar nichrome wire recording electrode (insulated except at the cut tip) in the fascia dentata (fd) (−3.3 from bregma, 2.5 lateral and 3.0 below the cortical surface). Reference and ground electrodes were 00-90 × 1/16" stainless steel screws threaded into the skull at 2 mm anterior to bregma and 2 mm right and left of the midline, respectively. Electrodes were connected to an amphenol receptacle, which was cemented to the skull with dental acrylic. Surgery was performed under 0.35 ml/100 g b.w. Chloropent anesthesia (Fort Dodge Labs.), with incisor bars elevated 5 mm above the interaural line.

Following a two week recovery period, recording studies were begun. At the time of recording the rats were placed in a small (8×20×38 cm high) recording chamber, and their amphenol headplugs were connected to Grass 7P511 amplifiers, with high and low pass filters set at 0.3 Hz and 3 KHz, respectively. Amplified signals (×500) were lead to an oscilloscope for monitoring and a PDP 11/70 computer for averaging and storage. The pp electrode was stimulated at 0.5 Hz, with 0.1 msec biphasic constant current square wave pulses produced by a Grass S88 stimulator and two PSIU-6 constant current converters. Current level was increased in steps of 100 μA until the evoked field potential contained a visually determined maximum amplitude population spike, a process which usually required about 30 stimuli. The intensity which produced this maximal response was then used to produce 16 subsequent paired responses, which were averaged by the PDP 11/70 computer. Amplitude of the resulting population spike was estimated using cursor controls on a Tektronix 4054 graphics terminal to measure the excursion from the EPSP to the spike peak and back. These two values

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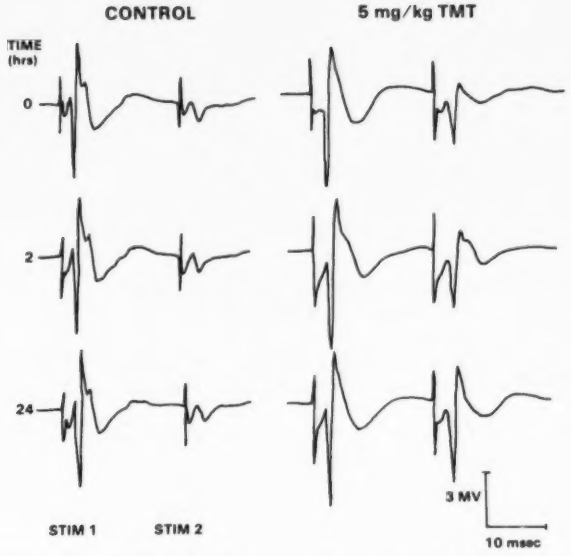

FIG. 1. Sample Recording from one saline and one 5 mg/kg TMT rat showing responses elicited by paired pulse stimulation of the perforant path immediately before and at 2 and 24 hours after treatment.

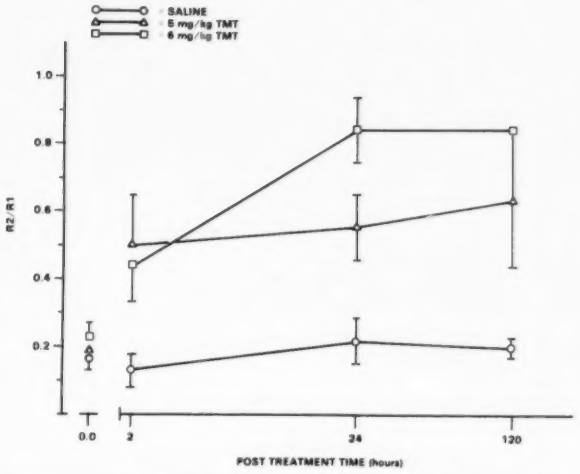

FIG. 2. Effects of TMT on recurrent inhibition. R1 is the amplitude of the population spike produced in the dentate gyrus by the first of the paired stimuli to the perforant path. R2 is the amplitude of the population spike produced by the second of the paired stimuli. The smaller the ratio of R2/R1, the greater the recurrent inhibition. Vertical bars are SEM. No bar is present on the 6 mg/kg 120 hr point, since this is a median value.

were added together and divided by two to obtain spike amplitude. This method is similar to that used by others (e.g., [11]).

Recurrent inhibition was assessed using the double pulse method described by Andersen *et al.* [1]. Briefly, the method entails presenting two identical stimuli at short interstimulus intervals (ISIs) to the pp electrode. At proper ISIs, the amplitude of the population spike recorded from the fd electrode following the second stimulus (R2) was reduced compared to the first (R1). Recurrent inhibition was expressed as the ratio of peak amplitudes (R2/R1).

In a pilot study, using different rats, the effect of ISI on magnitude of recurrent inhibition was assessed in unanesthetized and anesthetized rats. Subsequently unanesthetized rats were used throughout, and an ISI of 20 msec was selected for all studies.

Once appropriate stimulus parameters were obtained, each rat was administered either saline (n=14), 5 (n=12) or 6 (n=10) mg/kg TMT hydroxide as base by gavage in a volume of 1 ml/kg. These dosages were chosen because of the extremely steep dose-response function found with TMT [4,5]. Recording sessions were repeated 2, 24 and 120 hr after dosing. Groups were compared using repeated measures ANOVA, and comparisons between control and each dosage were made at each of the time points using Bonferroni-corrected [7] *t*-tests.

RESULTS

Prior to treatment, all three groups had equivalent recurrent inhibition, with mean amplitude of R2 averaging between 16 and 23% of R1. Trimethyltin produced an almost immediate decline in recurrent inhibition, as measured by the paired pulse technique. At the earliest time point (2 hr), R2/R1 was nearly twice what it had been in the predose test in TMT-treated rats. In contrast, the saline-treated rats maintained a consistent level of inhibition across the test times. Figure 1 illustrates the recordings obtained from a TMT-treated animal before and 24 hr following treatment. Figure 2 illustrates the averaged ratio R2/R1 at different post-treatment times. Due to death (n=2) and headplug dislodgement (n=4) only 4 of the 6 mg/kg treated rats had recordings made on post-treatment day 5. The median of these 4 scores is plotted in Fig. 2. The ANOVA indicated that the dose × time interaction was significant, $F(6,48)=28.16$, $p<0.0001$. Saline-treated rats were compared to each of the two TMT-treated groups at each of the four time points, for a total of eight comparisons. To maintain an experiment-wise error rate of 0.05, the required p value for each comparison was $0.05/8=0.00625$. Using this criterion, the 5 mg/kg TMT group differed significantly from the saline-treated group at the 2 hr ($p=0.0061$) and 120 hr ($p=0.0011$) post-treatment times, but not at the 24 hr post-treatment time ($p=0371$). The 6 mg/kg TMT group differed significantly from the saline-treated group at the 24 hr ($p=0.0006$) and 120 hr ($p=0.0001$) post-treatment times but not at the 2 hr post-treatment time ($p=0.0124$).

DISCUSSION

Administration of TMT reduced the size of the population spike recorded to the second stimulus of a pair, in a manner similar to what others have shown with bicuculline [11]. These findings suggest that TMT reduces recurrent inhibition in the dentate gyrus within 2 hr following dosing, and support the hypothesis that TMT increases activity in the mossy fiber system by reducing basket cell inhibition of the dentate granule cells. In this respect, TMT seems to be similar to kainic acid [10]. Based upon these findings, it is likely that the loss of CA3 pyramidal neurons in TMT-treated rats results at least in part from over stimulation.

This study raises several new questions. First, if TMT

selectively attacks the basket cells which are presumed to be responsible for recurrent inhibition, what is the basis for the selectivity? Possibilities include: (1) selective action against specific transmitter systems, including those used by basket cells; (2) a biochemical effect on all neurons with some being more sensitive than others because of unknown metabolic factors; (3) altered input to the basket cells (and/or other cells); (4) altered voltage-dependent conductance relationships; or (5) some combination of the above.

Second, can the loss of basket cell inhibition account for all of the TMT-induced damage reported in various studies? The answer to this question is almost certainly no. Neuronal damage has been described at sites which are either upstream from the dentate basket cells (e.g., the entorhinal cortex [3]), or outside the limbic system entirely [2]. Furthermore, there is evidence that in addition to release of inhibition, TMT may facilitate activity in certain synaptic systems [12].

Finally, while functional toxicity is not a new concept, occasions on which changes in neuronal activity are shown to be responsible for producing changes in brain morphology are relatively rare. This notion is not new in neuroscience (e.g., [6,9]) but it is relatively novel in the context of toxicology, and suggests new avenues for understanding mechanism of neurotoxicity.

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